Bio-Monolith QA Column

Data Sheet

About Bio-Monolith QA Columns

Bio-Monolith QA columns are high-performance monolithic columns that offer all the advantages of a specially designed, continuous short polymeric bed. Their inherent features enable highly reproducible separations of macro bio-molecules, large proteins, DNA, and viral particles, at extremely high speeds. These columns are prepacked in dedicated stainless steel housings and allow user-friendly (and quick) connections to HPLC equipment.

The information in this data sheet is being provided to ensure proper product care and maximal product lifetime.

Basic Characteristics

Catalog number	5069-3635
Column chemistry	Strong anion exchanger; quaternary amine ($P–N^+(CH_3)_3$) Counter ion: $Cl^–$
Matrix	Poly(glycidyl methacrylate -*co*- ethylene dimethacrylate) highly porous monolith
Ligand density	2.0 ± 0.2 mmol/g dry support
Matrix dimensions	Diameter: 5.2 mm; length: 4.95 mm; bed volume (CV): 0.10 mL
Dynamic binding capacity	≥ 25 mg BSA/mL wet support (Conditions: BSA 0.5 mg/mL, 20 mM Tris-HCl buffer, pH 7.4, flow rate 1 mL/min)
Maximum loading capacity	1.2 to 1.5 mg
Working flow rates	Recommended: 0.2 to 2 mL/min (1 to 10 cm/min; 2 to 20 CV/min) Maximum: 3 mL/min (15 cm/min; 30 CV/min)
Maximum allowed pressure over the column	150 bar (15 MPa, 2200 psi) **WARNING: Do not exceed the maximum allowed pressure as this might seriously damage your column!**
Temperature stability	Working: 4 to 40 °C (39 to 122 °F) Storage: 4 to 30 °C (39 to 73 °F) **WARNING: Avoid prolonged use at elevated temperatures!**
Recommended pH	Working range 2 to 13 Cleaning in place 1 to 14

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Taking Care of the Column

Regeneration

In order to regenerate the column (to wash any ionically bound substances off the column and to reintroduce the correct counter-ion), wash it with at least 2 mL (20 CV) of a buffer containing 1 M NaCl at 0.5 to 1.0 mL/min. Recondition the column with 5 to 6 mL (50 to 60 CV) of the starting (binding) mobile phase at 1 mL/min. For best results, repeat these steps at the end of each chromatographic run; it is recommended that you implement these steps within your method/sequence.

Cleaning in Place (CIP)

In some cases, simple regeneration of the monolithic column is not enough. Sample molecules may not fully elute from the column or may even precipitate on the column. This build up of contaminants on the monolithic column may cause loss of resolution and binding capacity, increased back pressure, or a complete blockage of the column. A specific CIP protocol should be designed according to the type of contaminants that are present in your sample. Two examples of the CIP procedure are presented; the first one can be used for regular CIP and the second for a more rigorous cleaning, where the contact time between the cleaning reagent (mostly NaOH) and the matrix is prolonged.

1. Regular cleaning

1.1 Wash the column with 1 to 2 mL (10 to 20 CV) of 1 M NaOH.

Note: Reverse the flow direction and use low-enough flow rates (0.2 to 0.5 mL/min) to expose the column to NaOH for several minutes.

1.2 Wash the column with 1 to 2 mL (10 to 20 CV) of deionized (DI) water at the working flow rate.

1.3 Wash the column with 1 to 2 mL (10 to 20 CV) of 1 M HCl at 0.2 to 0.5 mL/min.

1.4 Wash the column with 1 to 2 mL (10 to 20 CV) of DI water at the working flow rate.

1.5 Wash the column with 1 to 2 mL (10 to 20 CV) of 1 M NaCl at the working flow rate.

1.6 Re-equilibrate with at least 5 mL (50 CV) of the binding mobile phase (buffer) at the working flow rate.

If the impurities are highly hydrophobic or lipidic and are not easily removed from the column, you may also use other cleaning solutions, like 2-propanol, acetonitrile, or methanol (each up to 30%); acetic acid (30% to 50%); or guanidine hydrochloride (up to 6 M). If the main contaminant on the column is DNA tightly bound to the matrix, wash the column with a suitable enzyme solution (for example, DNA-se). After that, follow steps 1.2 through 1.6.

WARNING: When you wash the column with these cleaning solutions, always decrease the flow rate on the column in order to avoid back pressures that might exceed the maximum allowed pressure over the column.

For a more rigorous cleaning of the columns, the contact time between the cleaning reagent (mostly NaOH) and the matrix should be prolonged.

2. More rigorous cleaning

2.1 Wash the column thoroughly with 1 M NaOH for at least 1 hour at room temperature. Reverse the flow direction and pump NaOH through the column at the flow rate of 0.2 mL/min.

2.2 Alternatively, flush the column with at least 5 mL (50 CV) of the 1 M NaOH (reverse the flow direction), detach the column from the pump, seal with column end-stoppers, and leave in the solution overnight. Afterwards, attach the column to the pump and wash as previously described (see steps 1.2 through 1.6).

Long-Term Storage

If the column will not be in use for more than two days, it should be washed with at least 1 mL (10 CV) of DI water and afterwards flushed with at least 2 mL (20 CV) of a 20% ethanol solution at the flow rate of 0.2 to 0.5 mL/min, sealed with column end-stoppers, and stored appropriately (4 to 30 °C [39 to 73 °F]).

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